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Preparation of I^{131}-labeled Human Serum Prealbumin and Its Metabolism in Normal and Sick Patients *

EDWARD L. SOCOLOW, KENNETH A. WOEBER, ROBERT H. PURDY, MARY T. HOLLOWAY, AND SIDNEY H. INGBAR †

(From the Thorndike Memorial Laboratory, Second and Fourth [Harvard] Medical Services, Boston City Hospital, and the Departments of Medicine and Biological Chemistry, Harvard Medical School, Boston, Mass.)

Despite some early doubts concerning its presence in plasma and its physiological function (1–4), the thyroxine-binding prealbumin of human serum (TBPA) is now known to play a significant role in the transport of thyroxine (T_4) (5–8). Normally, as assessed by *in vitro* techniques, TBPA appears to bind at least 25 to 35% of T_4 in serum (5, 6). In the serum of many patients with severe acute or chronic illness, however, the proportion of endogenous T_4 in serum bound by TBPA and the T_4-binding capacity of TBPA decline (8, 9). The recent availability of substantial quantities of a highly purified preparation of TBPA has made possible an investigation of the *in vivo* metabolism of this protein in normal patients and in patients with a variety of disorders that lead to decreased binding of T_4 by TBPA in serum. In addition, the cause of this decrease in T_4 binding in the serum of such "sick" patients has been evaluated. A portion of the findings has been presented in abstract form (10). While these studies were in progress, Oppenheimer, Surks, Bernstein, and Smith reported similar studies in abstract form (11, 12).

Methods

Highly purified TBPA was prepared from plasma Fraction IV-6 (method 6) of Cohn and colleagues (13). The method of purification of the protein and details of its characterization will be described in detail elsewhere (14). Additional characterization of the specific batch of protein employed in the present studies was carried out. Solutions of TBPA (2.0 g per 100 ml), enriched with I^{131}-labeled T_4,[1] were subjected to electrophoresis in acrylamide gel in a Tris-maleate buffer system,[2] pH 9.2. Gel concentrations of 5.0, 7.0, and 8.0 g per 100 ml were employed, and, in some instances, two dimensional gel electrophoretic studies were conducted (15). After electrophoresis, gels were radioautographed and then stained with amido black 10 B.

Iodination of TBPA. To minimize the possibility of denaturing the TBPA during the iodination process, a technique was developed for iodinating protein by a microdiffusion process. The technique, an adaptation of that described by Banerjee and Ekins (16), was first employed in tests with human serum albumin (HSA) or with purified TBPA for relatively low specific activity labeling. When the method had been standardized, it was employed for higher specific activity labeling of the TBPA used in the present studies. This was performed as follows. All glassware and buffer media employed were autoclaved before use. A 2.0 g per 100 ml solution of TBPA was prepared in 0.01 M phosphate buffer, pH 7.5, and 200 μl was pipetted into the center well of a single side-armed Warburg flask of 5.0 ml capacity. NaI^{131} (10 to 15 mc, free of carrier iodide and reducing agent),[3] together with 60 μg of unlabeled NaI, was introduced into the main compartment of the vessel in approximately 1 ml of phosphate buffer. Five hundred μl of fresh 3% H_2O_2 [4] was added to the side arm, and the vessel was closed with a greased ground-glass stopper. The reaction was initiated by tipping the H_2O_2 into the main compartment, and the vessel was then transferred to a metabolic shaker at 37° C for 2 hours. After incu-

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† Address requests for reprints to Dr. Sidney H. Ingbar, Thorndike Memorial Laboratory, Boston City Hospital, Boston, Mass. 02118.

[1] The I^{131}-labeled thyroxine was obtained from Abbott Laboratories, Oak Ridge, Tenn.

[2] The Tris-maleate buffer employed in acrylamide gel electrophoresis was 0.018 mole per L in respect to both Tris and maleic acid. The buffer employed in filter paper electrophoresis was 0.073 mole per L in respect to both components.

[3] Obtained from Iso/Serve, Cambridge, Mass.

[4] Fisher certified reagent, Fisher Scientific Co., Boston, Mass.

bation, the vessel was opened, appropriate precautions being taken for the disposal of volatile radioactivity.

Preliminary experiments indicated that iodinations could be increased by allowing the protein to remain in contact at low temperature with the I^{131} that had distilled into the center well. Therefore, at the completion of incubation, the main compartment was emptied and the flask sealed and kept at −20° C for 24 hours. Thereafter, protein was removed from the center well, diluted in 20 ml of sterile phosphate buffer, and dialyzed overnight at 4° C against sterile isotonic saline solution. Sterile HSA was then added to a final concentration of 50 mg per ml, and this solution was passed through a Seitz filter before injection into patients. As a precaution against adsorption of TBPA to the filter pad, a sterile solution of HSA was passed through the filter before the TBPA.

Characterization of I^{131}-labeled TBPA. Preparations of I^{131}-labeled TBPA were analyzed for their content of total iodine and iodinated amino acids and for both electrophoretic mobility and T_4-binding capacity. Portions of the labeled protein solution were subjected to ascending filter paper chromatography in butanol-2 N acetic acid (BuAc, 1:1), butanol-dioxane-2 N ammonia (BDA, 4:1:5), and tertiary amyl alcohol-0.5 N ammonia (TAA, 3:1) solvent systems, both before and after hydrolysis with bacterial protease.[5] Localization of labeled components and determination of their relative proportions were carried out by scanning techniques described in detail elsewhere (17). The degree of iodination of the protein was calculated from the known specific activity of the iodide employed, the percentage yield of organic I^{131}, and an assumed molecular weight for TBPA of approximately 70,000 (18).

Electrophoretic mobility of the iodinated TBPA was assessed by scanning and radioautography after both filter paper and gel electrophoresis.

To ascertain the effect of the iodination procedure on the binding of T_4 by TBPA, a sample of TBPA was iodinated by the procedure described above, and another was retained as control. Both solutions were then enriched with HSA (2.5 g per 100 ml), with high concentrations of labeled T_4, and with concentrations of stable T_4 ranging between 3,000 and 5,000 μg per 100 ml. Solutions were subjected to filter paper electrophoresis concurrently, and calculations of T_4-binding capacities were carried out by conventional methods (19).

Studies of the metabolism of I^{131}-TBPA in vivo. Studies of the *in vivo* metabolism of I^{131}-TBPA were conducted in three categories of patients: A, four "hospital normals"; B, four essentially normal patients before and after the acute stress of surgery or administration of pyrogen; and C, three patients with chronic illness. All patients considered normal (i.e., categories A and B) were in good health at that time, and their sera had been found to have normal T_4-binding capacities of TBPA by conventional filter paper electrophoretic techniques. The three patients with chronic illness in category C had diagnoses of mild hepatic cirrhosis, severe cirrhosis, and prostatic carcinoma, respectively. The T_4-binding capacity of TBPA in their sera had been found to be subnormal in all.

Before injection of I^{131}-TBPA into the patients, the content of inorganic I^{131} in the solutions administered was determined by both ascending filter paper chromatography and filter paper electrophoresis (20). In some cases, solutions of I^{131}-TBPA were also dialyzed at 4° C against phosphate buffer, and radioactivity in the dialyzate was determined at 2 and 24 hours.

Twenty-four hours before injection, patients were given Lugol's solution, 10 drops three times a day, and this was continued throughout the period of study. Each patient received a single iv injection containing from 1.0 to 1.3 mg of TBPA labeled with 25 to 63 μc of I^{131}. The precise dose administered was determined by weighing syringes before and after injection. Multiple blood samples were obtained during the first 24 hours after injection, and daily samples were obtained thereafter. Twenty-four-hour urine collections were made in the eight patients studied on the metabolic ward; in only six studies, however, were collections considered to be nearly complete. In three patients, radioactivity was determined in the region of the liver, spleen, heart, neck, and femoral triangle by external scintillation counting.

In five studies in which patients were not subjected to acute stress and in the three chronically ill patients, observations were made for 10 to 15 days, except in patient J.K., in whom studies were conducted for only 8 days. Two volunteers among the normal patients were given bacterial pyrogen, and two patients underwent elective surgery after 6 to 7 days of control observations.[6] Observations were continued for approximately 1 week after the acute stressful stimulus. In these patients, measurements of the T_4-binding capacity of TBPA were made by conventional techniques before and at frequent intervals after the stressful stimulus.

The fractional rate of turnover (k) of I^{131}-labeled TBPA was determined from the exponential slope of decline in serum radioactivity, as calculated by the method of least squares (21). Data obtained during the first 48 hours after injection were omitted from statistical analyses to allow for thorough mixing of the labeled protein. In one patient in whom mixing appeared to be delayed (patient B.M.), data obtained during the first 72 hours after injection were excluded from analysis. Where applicable, fractional turnover rates were determined for both control and poststress periods. The volume of dis-

[5] Pronase, B grade, California Corporation for Biochemical Research, Los Angeles, Calif.

[6] The two volunteers who received bacterial pyrogen were given 25 million killed typhoid bacilli iv one day and 50 million bacilli the next. Transitory febrile responses resulted. The first patient subjected to surgery (B.M.) was a 32-year-old female with treated diffuse toxic goiter who underwent subtotal thyroidectomy. The other surgical patient (G.L.) underwent elective hemorrhoidectomy. Blood loss in both cases was negligible, and transfusions were not required.

tribution of I^{131}-TBPA in control studies was determined by a method previously employed to study the metabolism of radioactive T_4 *in vivo,* which is designed to correct for disproportionate loss of labeled material during the mixing phase (22). Since this calculation requires complete urine collection, volumes of distribution of I^{131}-TBPA are presented only for those studies wherein urine collections are thought to be adequate. Clearance rates were calculated as the product of the distribution space and fractional turnover rate, and ultimate urinary excretion of radioactivity (U_{max}) was calculated by methods previously described (22).

The electrophoretic mobility of the labeled materials that remained in the patients' sera was determined in five normal patients, including the four who underwent surgery or pyrogen administration. In the latter four patients, such determinations were made in sera obtained before and 3 days after the initial stressful stimulus. Because of the low levels of radioactivity present in such sera, a technique described in detail elsewhere, which permitted filter paper electrophoretic analysis of large volumes of serum (2.0 ml), was employed (23).

To determine whether the reduction in T_4 binding by TBPA in acute and chronic illness was due to displacement of TBPA from its usual electrophoretic locus, I^{131}-TBPA was added to sera obtained from two patients with chronic illness and to both pre- and poststress sera from the four patients who either underwent surgery or received pyrogen. Sera enriched in this manner were subjected to filter paper electrophoresis, and the localization of I^{131}-TBPA was determined by radioautography and strip-scanning.

In the patients rendered acutely ill, both control and poststress sera after decay of the radioactivity in I^{131}-TBPA were enriched with I^{131}-T_4 and were subjected to electrophoresis in acrylamide gels. Binding of labeled T_4 was assessed by radioautography.

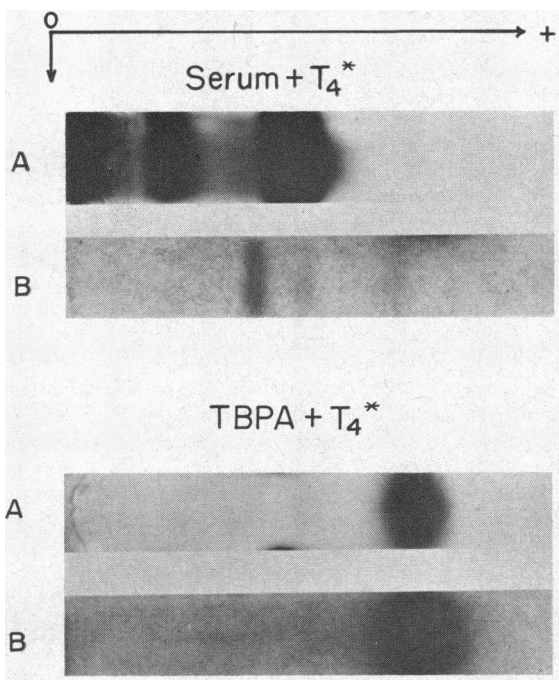

FIG. 1. ACRYLAMIDE GEL ELECTROPHORESIS OF NORMAL HUMAN SERUM AND OF PURIFIED THYROXINE-BINDING PREALBUMIN (TBPA), BOTH ENRICHED WITH I^{131}-LABELED THYROXINE (T_4*). A, protein stain; B, radioautograph.

Results

Characterization of purified TBPA. Gel electrophoresis of the radioactive T_4-enriched protein, later iodinated and administered to patients, revealed that the major component bound T_4 avidly and migrated to the position of prealbumin 1 of serum (24) (Figure 1). A minor component, not well visualized in photographs, migrated slightly faster than albumin, but well behind the major component, and also bound T_4. Two-dimensional electrophoresis demonstrated marked retardation of the mobility of the minor component in an 8.0% gel. This suggests that the minor component is of higher molecular weight than TBPA. Since this component binds T_4 and since it consistently appears during re-electrophoresis of TBPA eluted from gels, it most likely represents a polymer of this protein, and the mixture of the two will henceforth be referred to merely as TBPA.

Characterization of the iodination reaction. After 2 hours of incubation, 30 to 40% of the I^{131} had distilled from the main compartment into the center well of the Warburg vessel. Of this, only 3 to 10% had iodinated protein, as indicated by both paper chromatography and electrophoresis. After 24 hours at − 20° C, an additional 5 to 10% of the I^{131} in the well had become organified. Thus, over-all iodination yields ranged between 2 and 8%,[7] corresponding to iodine : protein molar ratios of less than 0.5 : 1.

Paper chromatography of dialyzed, unhydrolyzed specimens of I^{131}-TBPA revealed mainly immobile, organic I^{131} and small amounts of inorganic I^{131}; no free labeled amino acids were detected.[8] After hydrolysis of the I^{131}-TBPA, la-

[7] Iodination yields were consistently greater during experiments with HSA. In four experiments, over-all yields averaged 24%. The reason for this pronounced difference in the ease of iodination of HSA and TBPA is not known.

[8] In experiments with HSA, enrichment with unlabeled T_4 to a T_4 : HSA molar ratio of 1 : 1 or more was required to demonstrate labeling of the T_4 during protein

beled monoiodotyrosine was the only iodinated amino acid found.

Characterization of I^{131}-TBPA. Radioautography of a gel electrophoresis of I^{131}-TBPA mixed with HSA revealed that the main radioiodinated component migrated to the position of prealbumin 1 of serum. A variable, but very minor, proportion of the radioactivity migrated in the area of the presumed polymer. In filter paper electrophoresis, radioiodine was found only in the TBPA zone, and no minor iodinated component could be detected. No transiodination from TBPA to the HSA vehicle could be demonstrated in either electrophoretic system.

The T_4-binding capacity of I^{131}-TBPA was 3,544 μg per g protein, essentially unchanged from a binding capacity of 3,740 μg per g in a noniodinated portion of the same TBPA preparation (Figure 2).

iodination. Even under these conditions, only 3% of the I^{131} appeared as T_4; the remainder consisted of I^{131}-labeled protein.

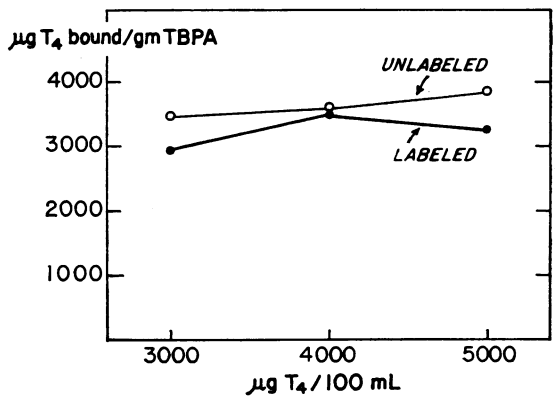

FIG. 2. COMPARISON OF THE THYROXINE-BINDING CAPACITIES OF TBPA BEFORE AND AFTER IODINATION WITH I^{131} AND I^{127}.

TABLE I

*The metabolism of I^{131}-labeled thyroxine-binding prealbumin in normal patients before and after acute stress and in chronically ill patients**

Patient	Age	Sex	Diagnosis	Body wt	BSA	T_4-binding capacity TBG	T_4-binding capacity TBPA†	Fractional‡ turnover rate	I^{131}-TBPA metabolism Volume of distribution§	Clearance	U_{max}
				kg	*m²*	*μg/100 ml*		*%/day*	*L*	*L/day*	*% dose I^{131}*
Normal patients											
P.B.	47	F	Normal	62.0	1.70	27.5	189	21.7 ± 0.3	7.1	1.5	75.9
J.B.	44	M	Normal	82.1	2.10	22.6	151	27.7 ± 0.4	9.8	2.7	61.5
J.K.	55	M	Normal	55.0	1.66	21.3	129	28.6 ± 0.5			
C.D.	57	M	Normal	52.0	1.56	26.9	128	19.1 ± 0.3	9.6	1.8	79.6
L.N. (a)‖	58	M	Normal	73.1	1.87	26.9	112	29.6 ± 0.5	12.1	3.6	76.4
L.N. (b)	58	M	Normal	73.1	1.87	27.0	108	33.2 ± 0.7	10.9	3.6	81.1
			iv Pyrogen			29.0	33	30.3 ± 0.1			
W.C.	40	M	Normal	55.1	1.61	26.0	127	29.4 ± 0.8	9.0	2.6	92.5
			iv Pyrogen			27.3	45	28.3 ± 0.1			
B.M.	32	F	Normal (treated thyrotoxicosis)	52.3	1.59	22.1	113	23.4 ± 0.1			
			Thyroidectomy			22.7	27	16.0 ± 0.6			
G.L.	42	F	Normal	62.7	1.62	23.0	128	33.0 ± 0.6			
			Hemorrhoidectomy			22.6	45	21.2 ± 0.6¶			
				Normal mean**		24.6	134	26.8	9.4	2.4	77.6
				SD		2.5	25	4.9	1.6	0.8	11.1
Chronically ill patients											
H.G.	79	M	Prostatic carcinoma, estrogen prescribed	54.0	1.61	41.3	45	22.1 ± 0.7	8.1	1.8	74.9
E.W.	55	M	Cirrhosis	63.1	1.70	33.2	38	23.3 ± 0.5			
N.T.	58	M	Cirrhosis	65.0	1.71	31.2	87	13.8 ± 0.4			
					Mean	35.2	57	23.1			

* T_4 = thyroxine; TBG = thyroxine-binding globulin; TBPA = thyroxine-binding prealbumin; U_{max} = ultimate urinary excretion of radioactivity.

† Values shown for postoperative or postpyrogen studies are the lowest values obtained, and all occurred on the third day after the stressful procedure.

‡ Values presented are mean ± standard error, as determined from the serum I^{131} disappearance curve by the method of least squares (21).

§ Because of the method of their measurement (22), volumes of distribution are presented only for those patients in whom urine collections were considered adequate.

‖ (a) indicates repeat study performed in patient LN after study (b) had been performed. In the calculation of mean ± standard deviation for the several functions shown in the normal group, the two control values obtained in LN were averaged and the average was considered as a single observation.

¶ Significantly different from control value; $p < 0.05$.

** Mean and standard deviation calculated during control periods only.

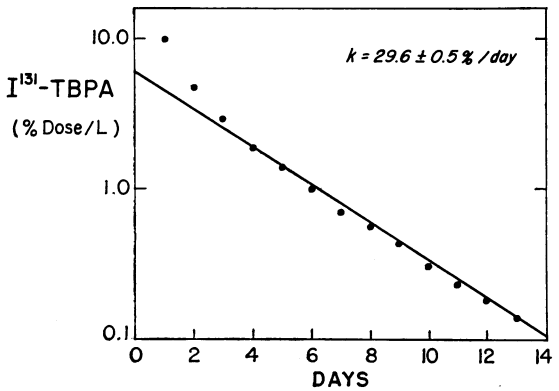

FIG. 3. DISAPPEARANCE OF A SINGLE INTRAVENOUS DOSE OF I^{131}-LABELED TBPA FROM THE SERUM OF A NORMAL PATIENT (L.N.). k = fractional turnover rate.

Inorganic I^{131} in the protein solutions administered to the patients ranged between 1 and 6% of total radioactivity, as assessed by multiple techniques, including dialysis. In no instance did dialyzable radioactivity increase when dialysis of protein solutions was continued beyond 2 hours, indicating that small iodinated molecules, such as polypeptides, were not contained in the injection mixture.

Metabolism of I^{131}-TBPA in vivo (Table I). After an initial mixing phase, I^{131}-TBPA was removed from the circulation at a rapid rate (Figure 3). In eight normal patients, the fractional turnover rate (k) averaged 26.8 ± 4.9% per day (mean ± SD). In the six patients in whom urine collections were thought to be complete, volumes of distribution of TBPA averaged 9.4 ± 1.6 L, and clearance rates averaged 2.4 ± 0.8 L per day. External counting revealed no evidence of hepatic concentration of I^{131}-TBPA, and radioactivity in the region of the liver, spleen, heart, and thigh declined at the same rate as that in the serum. In five normal patients, calculated values for the ultimate urinary excretion of I^{131} (U_{max}) averaged 77.6 ± 11.1% of the administered quantity.

In all patients subjected to acute stress, T_4-binding capacities of TBPA declined greatly, lowest values occurring on the third day after the stress. In the two patients who received pyrogen, both during the time in which the T_4-binding capacities of TBPA in sera were decreasing rapidly and during the subsequent period in which binding capacities returned toward normal, the fractional turnover rates of I^{131}-TBPA were unchanged from their control values (Figure 4). In the two patients who underwent surgery, fractional turnover rates for I^{131}-TBPA also failed to increase during the period in which T_4-binding capacities of TBPA were decreasing rapidly. Indeed, in both patients a slight decrease in fractional turnover appeared to occur, but in only one

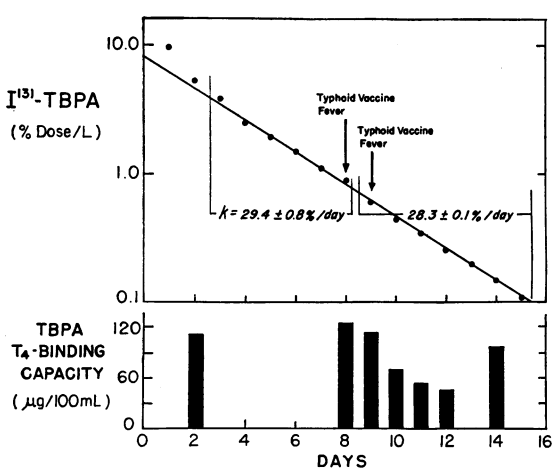

FIG. 4. THE EFFECT OF FEVER INDUCED BY TYPHOID VACCINE ON THE TURNOVER AND THYROXINE-BINDING CAPACITY OF TBPA IN THE SERUM OF A NORMAL PATIENT (W.C.).

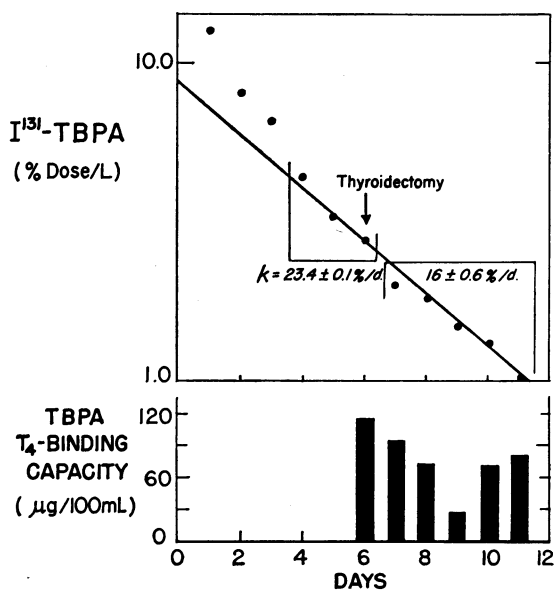

FIG. 5. THE EFFECT OF SURGICAL STRESS ON THE TURNOVER AND THYROXINE-BINDING CAPACITY OF TBPA IN THE SERUM OF A PATIENT WITH TREATED THYROTOXICOSIS (B.M.).

patient was this change of possible statistical significance ($p < 0.05$). This apparent slowing of turnover may have been the result merely of calculating turnover rates by the method of least squares, since in both patients, radioactivity in the serum on the first postoperative day was inordinarily low, whereas subsequent values conformed closely to those expected from a projection of the control disappearance curve (Figure 5).

In five normal patients, including four before subsequent stress, I^{131}-labeled components in serum were found to retain the rapid anodal mobility of the I^{131}-TBPA that they had been given, and this mobility was also unchanged in sera obtained after stress at a time when T_4-binding capacities of TBPA were decreased (Figure 6).[9] As in the case of I^{131}-TBPA contained in the patients' sera, I^{131}-TBPA added into control and poststress specimens from the four patients also retained its characteristic electrophoretic migration anodal to albumin (Figure 7). However, for reasons that are not clear, preparations of purified prealbumin, whether iodinated or labeled with T_4, tended to migrate slightly more rapidly than the endogenous TBPA in serum.

Gel electrophoresis of serial pre- and poststress sera in these four patients revealed a marked decrease in the density of the stain in the zone of prealbumin 1 during the period in which the T_4-binding capacities of TBPA were decreased. The density of the stained area corresponding to prealbumin 2 (24), a protein devoid of T_4-binding activity (25), was increased in some specimens, but not in all.

Fractional turnover rates of I^{131}-TBPA in the three patients with chronic illness and decreased T_4-binding capacities of TBPA were not accelerated. Rather, rates were slightly less than normal, averaging 23.1% per day. This slight difference from normal was not significant, but might have become so had a larger number of patients been studied. Paper electrophoresis of I^{131}-TBPA added to the sera of patients H.G. and E.W. demonstrated complete localization of radioactivity within the usual prealbumin area. Binding of T_4 by the thyroxine-binding globulin

[9] Zones of apparent slight radioactivity were occasionally seen in other areas of the electropherogram. In view of their variable location and minimal counting rate, these were considered to be artifacts.

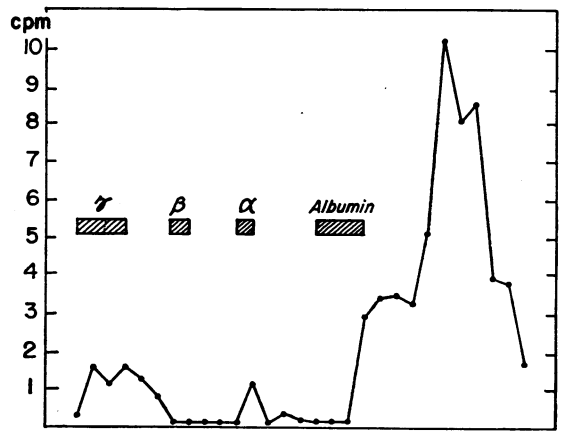

FIG. 6. ELECTROPHORETIC MIGRATION OF PREVIOUSLY ADMINISTERED I^{131}-LABELED TBPA IN THE SERUM OF A NORMAL PATIENT WHO HAD RECEIVED TYPHOID VACCINE (L.N.). Shaded blocks indicate electrophoretic migration of major protein groups. The serum studied revealed a marked decrease in the thyroxine-binding capacity of TBPA.

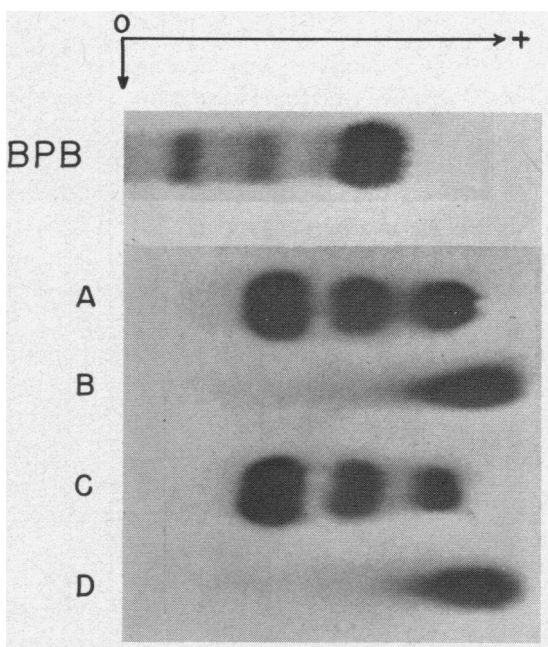

FIG. 7. ELECTROPHORETIC MIGRATION IN FILTER PAPER OF I^{131}-LABELED TBPA ADDED TO THE SERA OBTAINED FROM A NORMAL PATIENT (W.C.) BEFORE AND AFTER ADMINISTRATION OF BACTERIAL PYROGEN. BPB, protein stain of electropherogram. Other patterns shown are radioautographs of electropherograms. A, control serum + I^{131}-labeled T_4; B, control serum + I^{131}-labeled TBPA; C, postpyrogen serum + I^{131}-labeled T_4; D, postpyrogen serum + I^{131}-labeled TBPA. Thyroxine-binding capacity of postpyrogen serum was greatly decreased from that in control serum.

(TBG) was moderately increased in the serum of patient H.G., who was receiving estrogens for prostatic carcinoma, and was slightly increased in two patients with hepatic cirrhosis.

Discussion

As has been emphasized earlier (26, 27), the validity of utilizing isotopically labeled purified protein as a measure of the metabolism of its endogenous counterpart rests upon a number of assumptions. Among these is the assumption that the protein has not been altered from its native state by preparative procedures, by labeling techniques, or by subsequent self-irradiation. In the case of the TBPA used in the present studies, this assumption is supported by several lines of evidence. Although none is in itself conclusive, together they suggest that the metabolism of the I^{131}-TBPA studied should closely approximate that of endogenous protein. First, studies in several media indicated that the purified TBPA employed retained the electrophoretic migration characteristic of the native protein. Second, studies reported herein, and others described elsewhere (14), reveal that this preparation of TBPA retained essentially the same T_4-binding capacity (3,740 μg T_4 per g protein) as the endogenous protein in serum (approximately 120 μg T_4 per 100 ml in this laboratory) if, as has been suggested, the concentration of the protein in serum is approximately 30 mg per 100 ml (18). To minimize the possibility that the iodination procedure would alter the protein, a method for iodination was employed that is considered to reduce such hazards greatly. In this method, the protein is never in direct contact with organic solvents, with oxidants other than the reactive form of iodine that carries out the iodination, or with reductants that are employed in some methods to stop the iodination reaction. Furthermore, the iodine : protein molecular ratio achieved was purposefully kept low (less than 0.5 : 1), and only relatively small amounts of radioactivity were introduced. Data obtained with other proteins indicate that far heavier iodination and more extensive irradiation than occurred in the present preparation are required to alter their *in vivo* metabolism (26, 27). It seems significant, therefore, that the labeled protein and the unlabeled material did not differ detectably with regard to electrophoretic mobility or T_4-binding capacity. Furthermore, turnover rates of I^{131}-TBPA were remarkably uniform in the normal patients, despite the fact that 4 different batches of iodinated protein were administered to this group. In patient L.N., moreover, repeat studies with different batches of protein provided values that agreed closely. Finally, when turnover studies were carried out in the normal patients for 15 days, i.e., at least 4 half-lives after apparent mixing, no evidence of a multicomponent system could be detected. Because supplies of purified TBPA are relatively limited, it has not been possible to study the *in vivo* metabolism of I^{131}-TBPA prepared by varying techniques and containing varying quantities of I^{127} and I^{131}, as has been done in the case of iodoalbumin and iodoinsulin (26–28). Therefore, within the limits of current practicability, it is considered that the I^{131}-TBPA herein employed constitutes an adequate tracer for the endogenous protein.

Studies with I^{131}-TBPA indicate that in normal adults the protein distributes in a volume of approximately 9 L. As assessed by external counting techniques, preferential accumulation of the protein in the liver or other viscera does not occur. The volume of distribution of TBPA does not differ appreciably from that of HSA (26) and that of T_4 (22). If the volume of distribution of TBG, the other major thyroxine-binding protein of serum should prove to be much like that of albumin and TBPA, the close concordance of these volumes would suggest that only a very small proportion of extrathyroidal T_4 is present in exchangeable loci within the cells themselves. A similar conclusion has been drawn from observations indicating that pronounced changes in the extracellular binding of T_4 are not accompanied by measurable alterations in the total volume of T_4 distribution (29). If then, only a small proportion of peripheral T_4 is affixed to the cells, the turnover of cellular T_4 must be rapid, since the total pool of T_4 turns over at a rate of approximately 10% per day (22, 30).

Fractional rates of turnover of I^{131}-TBPA in normal patients were both rapid and remarkably uniform, averaging 26.8 $\pm$ 4.9% per day (mean $\pm$ SD). Corresponding values for the half-time of I^{131}-TBPA in the plasma were 2.67 $\pm$ 0.53 days, values in close agreement with those reported by Oppenheimer and colleagues for the terminal slope

of I^{131}-TBPA disappearance (12). Thus, TBPA appears to be among the most rapidly turning over of the plasma proteins thus far studied (26, 31–34).

Mathematically extrapolated values for the ultimate urinary excretion of I^{131} derived from the labeled TBPA averaged $77.6 \pm 11.1\%$. If urine collections are assumed complete, then a portion of the I^{131} label must have been lost by other routes. Since thyroidal accumulation of I^{131} was blocked by administration of Lugol's solution, inorganic I^{131} should have been lost in the urine. Therefore, excretion of organic products of I^{131}-TBPA metabolism via the gastrointestinal tract seems the most likely explanation for the radioactivity that was not recovered in the urine.

It has been clearly demonstrated that the T_4-binding capacity of TBPA is often decreased in the serum of patients with one of a number of acute or chronic systemic disorders (8, 9, 29). The availability of purified I^{131}-labeled TBPA has made possible an inquiry into the factors responsible for this change in the T_4-TBPA interaction. Several possible causes of a decrease in the binding of T_4 in the prealbumin zone of electropherograms suggest themselves. First, an inhibitor of T_4-binding by TBPA may appear in the serum. Second, TBPA may undergo an interaction with other proteins or components of the plasma with the result that it no longer migrates to its characteristic electrophoretic locale. Third, the concentration of the protein in the plasma may actually decrease. Obviously, a combination of the foregoing mechanisms could occur. Each of these possibilities and the pertinent data will be discussed in turn.

In studies to be published elsewhere, no evidence for the presence of an inhibitor of T_4-binding by TBPA in the serum of sick patients could be obtained (35). Thus, dialysis of normal serum against serum with decreased T_4 binding by TBPA did not decrease the T_4-binding capacity of the former or increase the capacity of the latter. Furthermore, in varying mixtures of sera with normal and subnormal T_4-binding capacities of TBPA, the resulting binding capacities were those to be expected from the original binding capacities of each. Had an inhibitor of binding been present in excess in sera in which binding by TBPA was decreased, recovery of T_4-binding capacity would have been less than expected. Furthermore, the increase in the binding capacity of TBPA induced by adding the purified protein to normal and abnormal sera was equal. Finally, a simple inhibition of T_4 binding would not explain the observation by Oppenheimer and co-workers, confirmed in the present studies, that the density of the protein stain is decreased in the prealbumin zone of gel electropherograms of sera from sick patients (8, 36).

Such decreased density of protein staining in the prealbumin zone of the serum of sick patients would occur if the electrophoretic migration of TBPA were altered. Any such alteration in migration would necessarily require that the binding activity of the protein also be reduced, since the T_4-binding activity that is lost from the prealbumin zone in such sera is not recovered elsewhere. Of greatest importance in this regard, however, are the present findings that in the sera of acutely or chronically ill patients, no alteration occurred in the migration of the I^{131}-TBPA present as the result of either previous administration of the labeled protein or its direct *in vitro* addition.

Exclusion of the foregoing possible factors indicates that the decreased T_4-binding by TBPA in the serum of sick patients is due to an actual decrease in the concentration of the protein, a conclusion with which the decreased protein stain in the prealbumin area would also be consonant. Such a decrease in protein concentration could result from decreased synthesis or from enhanced removal of the protein, through proteolysis, sequestration, or excessive excretion. The present studies seem clearly to exclude enhanced removal of TBPA as the causative factor. In four patients subjected to sufficient acute stress to decrease the T_4-binding capacity of TBPA greatly, no increase in the rate of disappearance of I^{131}-TBPA from the serum was evident during the period in which T_4 binding by TBPA was changing. Furthermore, in chronically ill patients in whose sera T_4 binding by TBPA was also decreased, the fractional turnover of I^{131}-TBPA was decreased, rather than increased. These findings implicate decreased synthesis of TBPA as the cause of its decreased concentration in the serum of acutely and chronically ill patients.

Consistent with this interpretation are the observations that the rate of loss of T_4-binding ca-

pacity of TBPA in acutely ill patients is of the order of magnitude of the rate of turnover of the protein. The proximate cause of the apparent decrease in TBPA synthesis that often is evident in acute and chronic illness is unclear, and it is not known whether this is part of a more general response, since the effect of stress on protein synthesis is variable and uncertain (37).

Both direct measurement and indirect indexes reveal that the proportion of T_4 that is unbound or free is increased in the serum of many acutely or chronically ill patients (8, 38). This change has been correlated with decreased binding of T_4 by TBPA and is apparently associated with an increased rate of peripheral turnover of T_4 *in vivo* (39, 40). If the latter change does reflect an increased requirement of peripheral tissue for T_4, then the decrease in T_4 binding by TBPA, the origin of which has been discussed above, would appear to have adaptive value. Such a mechanism would be consistent with the postulated role of TBPA as a physiologically labile source of T_4-binding sites (6) and would be well served by the rapid turnover of protein herein described, since an inhibition of TBPA synthesis would rapidly result in a decrease in total T_4-binding sites and an increase in hormone available to the cells. This would not be the case if the turnover of TBPA were normally more prolonged.

Summary

A preparation of highly purified thyroxine-binding prealbumin was radioiodinated by a microdiffusion technique. Neither the electrophoretic mobility nor the thyroxine-binding capacity of the labeled protein (I^{131}-TBPA) differed from that of the starting material. Metabolism of I^{131}-TBPA was studied after intravenous administration in normal and sick patients. In normal patients, the distribution space of I^{131}-TBPA averaged 9.4 L and the turnover rate 26.8% per day. In four patients, the rate of disappearance of I^{131}-TBPA was not significantly altered after an acute stressful stimulus that caused the thyroxine-binding capacity of TBPA in their sera to decrease greatly.

The I^{131}-TBPA administered retained its characteristic electrophoretic mobility within the patients' sera, even in those poststress specimens in which the thyroxine-binding capacity of TBPA was decreased. This was also true of I^{131}-TBPA added directly to such sera. No abnormality in the metabolism of I^{131}-TBPA was evident in three patients with chronic illness in whose sera the thyroxine-binding capacity of TBPA was subnormal.

In view of the foregoing findings and of both the decreased density of protein stain in the TBPA zone in sera of sick patients and the evidence against the presence of an inhibitor of thyroxine-binding by TBPA in such sera, we conclude that decreased thyroxine-binding by TBPA in the sera of sick patients results from decreased synthesis of the protein.

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